

Application No. 10/734,609  
Amendment dated January 30, 2006  
Reply to Office Action of September 30, 2005

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### REMARKS

With the entry of the present Amendment, claims 1-26 and 28-29 are in this application. Claim 30 has been canceled without prejudice. No amendments are made herein.

#### The Telephonic Interview

Applicants express their appreciation to the Examiners for their time and thoughtful consideration of the issues in the telephonic interview held January 24, 2006. Participants included the undersigned agent for Applicants (Donna M. Ferber), inventor Jonathan Smith, Franco Salvoza and James Housel. Applicants presented their arguments concerning the prior art rejections, with supplementary scientific discussion of distinctions over the prior art provided by Jonathan Smith. The Patent Office verbally agreed that the prior art rejections had been overcome.

#### The Information Disclosure Statement

Applicants respectfully note that an electronic Information Disclosure Statement was filed by the undersigned on October 7, 2004, and that this submission was acknowledged on that date by the United States Patent and Trademark Office. Paper copies of the Acknowledgement and the Information Disclosure Statement were provided with the response to the previous Office Action. The Examiner still has not provided an initialed copy of that submission to acknowledge that those US patents and published applications have been considered.

Applicants again respectfully request that the Examiner consider the listed United States patent references and that he initial and return a copy of the list of United States patent references cited on the electronic Information Disclosure Statement.

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The Rejections under 35 U.S.C. 103(a)

The rejection of claims 17, 24 and 27 under 35 U.S.C. 103(a) as allegedly obvious over Pushko et al. (1997) has been maintained. Applicants respectfully traverse this rejection.

The Pushko reference is said to teach methods of preparing alphavirus replicon particles (ARPs) comprising introducing an alphavirus replicon vector and one or more helper nucleic acid molecules into alphavirus-permissive cells via electroporation. The Patent Office has conceded that the cited reference does not teach a specific concentration range of permissive cells during electroporation or the concentration of the replicon vector. The Examiner has alleged that "one would know that such concentrations can be optimized".

As noted above, the Patent Office has acknowledged that the Pushko reference does not teach the concentrations of cells and nucleic acid recited in the present claims, nor does there appear any suggestion to improve particle yield (or the economy of particle production) by altering the cell or nucleic acid concentrations in the electroporation step in any direction, let alone by altering in any particular direction or by using the concentration ranges currently claimed. Moreover, there is no reasonable probability of success provided in the cited Pushko reference for the changes made by the present Applicants. In re O'Farrell, 7 U.S.P.Q. 2d 1673, C.A.F.C. 1988, requires that a reference cited as making a claimed invention obvious must provide that reasonable probability of succession in the claimed invention. However, Applicants respectfully urge that in situations like the present invention, what would have been obvious to try would have been to vary all parameters to try each of numerous possible choices until one possible arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be success. The required reasonable predictability of success is not provided for in the reference.

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In the as-filed Specification in the paragraph bridging pages 23 and 24, the typical cell density range in electroporation mixtures is discussed.

Additionally, the density of the cells is also a factor, and manufacturers' recommendations are between  $1-5 \times 10^6$  cells/mL for Vero and NIH-3T3 cells, and slightly higher for BHK and CHO cells, both of which are smaller than Vero cells (see, e.g. Multiporator® Cuvette Manual, Brinkmann, Westbury, NY; Genetronics, San Diego, CA (BTX Division) protocols for the ElectroCell Manipulator (ECM®) or the ElectroSquarePorator™, Parham, J. et al. 1999 *CytoTechnology* 28:1-9). The art teaches that higher cell densities than those recommended result in non-homogenous field conditions in the electroporation milieu, which can lead to cell fusion. Liljestrom and Garoff, J. *Virology* 65:4107-4113, 1991, used electroporation to introduce a single, capped RNA helper and a Semliki Forest Virus replicon RNA into BHK cells at a concentration of  $5 \times 10^6$  cells/mL.

Thus, the art appears to teach the use of significantly lower cell densities than those specified in claims 17 and 24 (as renumbered), from which claim 28 (as renumbered) now depends. Accordingly, Applicants respectfully submit that the art in fact teaches away from the present invention as claimed.

Applicants respectfully maintain that there is no teaching or suggestion that particle yield could be or would be improved with the utilization of the particular host cell and nucleic acid concentrations specified in claim 17.

Neither does the cited reference teach or suggest that contacting the cells in which the viral particles were produced with an aqueous solution of from 0.2 to 5 M ionic strength as specified in step (c) of claim 1 and step (c) of claim 25, from which claim 27 now depends, could or would improve ARP yield. Further discussion of the reference is provided below.

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In view of the foregoing, Applicants respectfully maintain that the claims 17, 24 and 28 (formerly 27) are not made obvious by the teachings of the cited Pushko reference, and withdrawal of the rejection is requested.

Claims 1, 3-12, 14-27 and 29 have been rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Pushko in view of Bell et al. (1978). Applicants respectfully traverse this rejection.

The Patent Office has characterized the cited reference as teaching a replicon vaccine vector system based on an attenuated strain of VEE, where the replicon nucleic acid comprises at least a virus packaging signal and at least one heterologous coding sequence expressible in the alphaviral replicon nucleic acid, where the host cell comprises at least one helper function, to produce a modified host cells. In this case the helper functions were provided either by monopartite or bipartite helper systems. Pushko is said to teach culturing the modified host cell under conditions allowing expression of the at least one helper function, allowing replication of the alphavirus replicon nucleic acid and packaging of the alphaviral nucleic acid to form ARPs. The Patent Office has acknowledged that the cited Pushko reference does not teach the effect of salt concentration on the modified host cell after the culturing step to release the ARPS to produce an ARP-containing solution.

The Bell reference is said to teach the effect of salt concentration on the release of alphavirus particles in cell culture systems. Bell described results from cell culture systems used to replicate Sindbis virus. Bell reported that "when the NaCl concentration is lowered, **maturation of infectious particles and particle release** is significantly reduced". The importance of Bell is said to be that "it establishes a dependence between salt concentration and particle release at the point when particles are harvested in cell culture. While Bell does not explicitly teach an optimal salt concentration or range, it is alleged that "one would recognize from Bell that one should

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optimize the salt concentration because such optimization greatly affects virus particle yield."

As discussed in the telephonic interview by coinventor Jonathan Smith, the Bell paper states that under the low salt medium condition, the particles cannot be released from the cells by protease treatment; therefore, the particles are not on the cell surface. The Bell paper teaches that maturation of the PE2 precursor protein to the E2 protein is inhibited in low salt medium; it is known in the art that PE2 is cleaved during the virus maturation process. This is evident from the Bell reporting of PE2:E2 ratios. The cited Bell paper further reports that whereas anti-E1 and anti-E2 antisera prevent virus release from cells in normal medium, the anti-E1 sera, but not the anti-E2 sera, prevent release from cells in low salt medium. PE2 is not matured to E2, again indicating that the virus particles are not mature. In addition, radioiodination of virus-infected cells in normal medium efficiently labels E2, indicating that PE2 is not on the surface or not mature, in either event, indicating that particle maturation is inhibited in low salt medium and the particles are not on the surface of the cells. By contrast, in the present Specification, the cells producing the ARPs are incubated in medium of normal ionic strength, and the particles released in the high salt wash are completely matured, as reflected by the fact that they are fully infectious, neutralized by anti-E2 antibodies and can be completely released at 4 °C (the low temperature inhibits budding of the ARPs from the cell through the membrane).

Applicants also point to Figure 6 of the as-filed application; this figure demonstrates the dramatic effect of the salt wash step on ARP yield.

The Patent Office further concluded that one of ordinary skill in the art would have been motivated to combine the teachings of Bell and Pushko and that one of ordinary skill in the art would have expected an increased yield of non-infectious particles because the teaching were well-established at the time of applicant's invention.

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Pushko is further said to teach the use of Vero cells as the alphavirus-permissive cells. The Patent Office has added that it would have been obvious to optimize the electroporation mix and the gap between electrodes to ensure transfections of 100% of the cells through routine experimentation. As to claims 10 and 12, the Patent Office has alleged that one would reasonably wash the cells and would perform this wash in a medium of reduced salt to prevent premature release of virus, and one would also remove DNase to remove residual nucleic acid in the media. Applicants note that claim 12 relates to a DNase treatment. As to claim 5, per the Patent Office, this again appears to be a matter of routine optimization/experimentation. As to claim 22, which recites purification methods, one would reasonably filter or purify the virus following harvest. The Patent Office has also concluded that the use of capped vs. uncapped RNA transcripts would appear to be within the purview of routine experimentation.

Claims 1 and 26 (as renumbered) have now been amended to recite that the alphavirus replicon nucleic acid is of a heparin-binding alphavirus. This amendment is supported by the as-filed Specification, for example, at the paragraph bridging pages 28-29. Neither of the cited references makes a connection between the ability of a virus or ARP to bind to a heparin (or other glycosaminoglycan) and the improvement in virus or particle yield when the producing cells are subjected to a contacting/release step with a solution having an ionic strength from 0.2 to 5 M.

Applicants respectfully maintain that the cited Bell reference appears to be related to the effect of **growth** medium salt concentration (half-strength v. normal strength) on maturation of virus particles in infected cells, rather than the effect of higher than normal medium salt concentrations in washes **after** virus or particle propagation and maturation. The effect of salt in the present invention is not dependent on incubation temperature; thus, Applicants conclude that the salt does not stimulate maturation of ARPS, but rather that the contacting step removes (already) mature ARPs from the cells. In addition, it is noted that the Bell reference relates to incubation of the virus particle-producing cells in medium with half the normal concentration of NaCl and

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then further incubating those cells in medium containing the normal NaCl concentration. Applicants respectfully maintain that the combination of the teachings of the Pushko and Bell references would not have led one of ordinary skill in the art to the present invention as claimed. The courts have cautioned against the impermissible use of hindsight in evaluating patentability, and they have held that it is necessary that the cited references provide the motivation for their combination. See, for example, ACS Hospital Systems, Inc. v. Montfiore Hospital, Inc., 221 U.S.P.Q. 929, C.A.F.C., 1984; Northern Telecom, Inc. v. Datapoint Corp., 15 U.S.P.Q.2d 1321, 1323 (Fed. Cir. 1990); In re Oetiker, 24 U.S.P.Q.2d 1443 (Fed. Cir. 1992) ("[t]here must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination" and "[t]hat knowledge can not come from the applicant's invention itself."); and In re Dow Chemical Co., 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Neither reference teaches or suggests any washing of the virus- or particle-producing cells in a higher than normal medium salt concentration as a way to improve yield.

Applicants further note that at page 768 in the Bell reference, it is suggested that the virus particles associated with the cells were an artifact of the fixation process prior to electron microscopy – one of ordinary skill in the art would not be likely to consider any increased-salt washing treatments for particle preparation if the association was believed to be the result of the fixation step.

Moreover, one of ordinary skill in the art might well have expected that Bell would have suggested changes in salt concentration to higher levels if it was really thought that would improve yield or that the Bell research group would have experimented with the higher salt levels. However it is noted that elevated salt concentrations would likely have damaged the cells in which the virus particles were produced, and with maturation as a suspected problem, the ordinary skill artisan would not have been likely to increase the salt concentration during any step of virus production. Again, there is nothing which suggests that virus particles were adhered to cell surfaces in the absence of fixation so

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there would have been no motivation to wash them off with a medium or buffer of elevated salt concentration. There is no teaching or suggestion that increasing the ionic strength above "normal" concentration would give further virus release. It appears that the Examiner may be using the teachings of the present Specification to interpret the cited Bell reference with the impermissible benefit of hindsight.

In addition, Applicants point to Figure 4 of the present application, which shows that the salt wash can result in as much as a **hundred-fold** increase in ARP yield. Applicants respectfully submit that the magnitude of the increase in particle yield is significantly greater than one would expect from mere routine optimization of experimental parameters and that an increase in yield of this magnitude constitutes a surprisingly improved result.

In view of the foregoing, Applicants respectfully maintain that the Patent Office has not established that the claimed invention is *prima facie* case obvious over the cited references, and the rejection must be withdrawn.

Claim 2 has been rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Pushko in view of Bell as applied above and further in view of Polo et al. (1999). Applicants respectfully traverse this rejection.

Claim 2 specifies that the at least one helper function is encoded by a nucleic acid sequence incorporated within the genome of the host cells. The cited Pushko and Bell references do not teach this, but the cited Polo reference teaches such a cell. The Patent Office has concluded that one of ordinary skill in the art would have been motivated to apply the teachings of Polo and that the invention would have been obvious to one of ordinary skill in the art at the time the invention was made.

As argued above, neither the Pushko nor the Bell reference teaches or suggests that washing the cells (and/or cell debris) in which the viruses or particles with heparin-



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binding properties were produced with a solution having an ionic strength from 0.2 to 5 M has a beneficial effect on the virus or particle yield. As argued above, the Bell reference appears to teach a relation between virus maturation and low salt v. normal salt concentration in the culture medium. Again, the present invention entails an ionic strength dependent contacting/release step which is not dependent on temperature or on the presence of growth medium; accordingly, Applicants have concluded that it is a wash (release) rather than maturation. The present inventors have made the correlation between the heparin-binding ability and the improved yield with a salt release step. Neither does the cited Polo reference or either of the other cited references teach or suggest such a step, as required by base claim 1. In addition, while Bell teaches that lowering the salt concentration has a negative effect on particle maturation and yield, there is nothing that suggests that an increase in the salt concentration in a wash step would have a beneficial effect on yield. There is no reasonable provision of success in the salt wash step as taught in the present application. Therefore, the rejection for alleged obviousness is not proper and must be withdrawn.

Accordingly, Applicants respectfully maintain that claim 2 is not *prima facie* obvious over the cited references, and the rejection must be withdrawn.

#### Conclusion

Applicants respectfully submit that the pending claims are in condition for allowance and early notification thereof is requested.

If, in the interest of expediting prosecution, the Examiner has questions or comments, he is invited to telephone the undersigned at the indicated telephone number.

This Response is accompanied by a Notice of Appeal with Petition for Extension of Time (one month) with authorization therein for the payment of the \$120.00 extension

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fee and \$500.00 Notice of Appeal fee. It is believed that this Amendment does not necessitate the payment of any additional fees under 37 C.F.R. 1.16-1.17. If this is incorrect, however, please charge any fees due pursuant to the foregoing Rules to Deposit Account No. 07-1969.

Respectfully submitted,



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